Chapter 11
Chapter II

MATERIAL AND METHODS

Storage deterioration was studied mainly in relation to cereals and their products, viz., rice (Oryza sativa), wheat (Triticum sativum) and corn (Zea mays). Samples were collected from different storage places in Gauhati as well as from bakeries.

Fungi present in the storage atmosphere were isolated from various sources where cereals were stored or converted into flour or other products. The method adopted was by exposing petri plates. The plates were incubated and the various colonies isolated and identified. A colony counter was used to determine the number of colonies present.

Pure cultures were used for reinoculation of the various foodstuffs to determine their specificity. The media used during their investigation were Potato dextrose agar, Czapek's media and Sabourard's media.

Potato Dextrose agar was prepared with the following ingredients:

- Peeled Potato 250.0 g.
- Dextrose 20.0 g.
- Agar powder 15.0 g.
- Distilled water upto 1.0 litre
  pH adjusted to 6.0 to 6.5.

Czapek's media (1910) was prepared as follows:
Sucrose 30.0 g.
Sodium nitrate 2.0 g.
Potassium hydrogen phosphate 1.0 g.
Magnesium sulphate \((\text{MgSO}_4, 7\text{H}_2\text{O})\) 0.5 g.
Potassium chloride 0.5 g.
Ferrous sulphate \((\text{FeSO}_4, 7\text{H}_2\text{O})\) 0.01 g.
Agar powder upto 15.0 to 20.0 g.
Distilled water pH adjusted to 6.0 to 6.5

Sabouraud's media contained the following ingredients:

- Peptone (mycological) 10.0 g.
- Maltose or dextrose 40.0 g.
- Agar powder 15.0 g.
- Distilled water upto 1.0 litre

Knop's normal culture solution was used to study effects of toxins on seedlings and was prepared with following ingredients:

- Potassium nitrate \((\text{KNO}_3)\) 1.0 g.
- Acid potassium phosphate \((\text{KH}_2\text{PO}_4)\) 1.0 g.
- Magnesium sulphate \((\text{MgSO}_4)\) 1.0 g.
- Calcium nitrate \((\text{Ca(NO}_3)_2)\) 4.0 g.
- Ferric chloride solution \((\text{FeCl}_3)\) a few drops
- Water upto 1.0 litre

The moisture content of the cereal grains (rice, wheat and corn) was determined by Universal Oswa moisture meter.
The effect of temperature on the growth of fungi was determined according to the method followed by Cochrane (1958).

The relative humidity was calculated as per method described by Harvey (1948).

The total sugar was estimated by Somogyi's method (1945).

The toxic effects of infected foodstuff were tested on the following:

(A) Seed and seedlings of Mung (Phaseolus aureus),
    of Gram (Cicer arietinum),
    of Corn (Zea mays),
    of Wheat (Triticum sativum), and
    of Paddy (Oryza sativa);

(B) Embryonated eggs;

(C) One day old chick of Australorp strain; and also on

(D) Albino mice.

Extraction of toxin:

(A) Crude extracts were prepared as follows: 50 ml. of Czapek's media was mixed with 25 g. of rice powder and sterilized at 15 lb. pressure for 20 minutes. This was innoculated with different fungi, viz., A. flavus, P. notatum, and H. sativum. After 10 days of incubation the fungal mat was taken out, extracted with 100 ml. of sterile distilled water. This was concentrated to 40 ml. in hot water bath at 30°C.
Extracts were purified as per Zdena et al's method with slight modification, viz., using methanol instead of chloroform. Again the proportion of fungal mat and methanol used in this experiment was 1:4 instead of 1:1.

Identification and elution of the toxins were done by Thin Layer Chromatography (T.L.C.) method of Zdena et al (1976) as follows:

Thin layers of silica gel of 0.25 mm thickness were prepared on glass plates (20 x 20 cm). Plates were activated for 2 hours at 110°C. Before use, plates were cleaned by developing them in the system that was subsequently used for their development with samples. After evaporating the solvents plates were stored for 24 hours in desiccator with dilute Sulphuric acid. The relative humidity in the desiccator was about 40%.

The solvent system used for T.L.C. is given below:

(a) Chloroform : Methanol (4:1)
(b) Benzene : Methanol : Acetic Acid (24:2:1)
(c) Chloroform : Acetone (9:1)
(d) N Butanol : Acetic acid : water (4:1:4)

Solvent was allowed to run 10 cm in the plate.